Thesis title:

Preliminary *in silico* Docking Study with Selected FDA Approved Drugs for Neurological Disorders

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Summary

The current project focused on five FDA approved drugs that are prescribed to treat various neurological conditions. The drugs selected for the study include Memantine, Levodopa, Tetrabenazine, Gabapentin, Zolpidem. *In silico* docking methods were used to assign Ki values to the FDA approved drugs with the 10 different targets. The *in silico* study evaluated if these five drugs would bind to 10 targets that are not known to bind to the five drugs tested. The targets selected include 5HT5 receptor, 5HT4 receptor, α7 nicotinic acetylcholine receptor, α4β2 nicotinic acetylcholine receptor, D4 receptor, monoamine oxidase A (MAO-A), monoamine oxidase B (MAO-B), $GABA_A$, $GABA_B$, and calcium-sensing receptor (CaSR). The results from the study concluded that all drugs in this study have an interaction with at least one of these 10 targets. These off-target interactions could be useful in discovering if they are the result of the drug's side effects, or if they are part of the drug's mechanism of action. The data from this study could also be useful for repurposing the drugs to treat other conditions. Further research needs to confirm if any of the significant interactions are real, and what type of change they cause in the body.

Background

Small molecule drugs have been shown to bind to more than one receptor, transporter or enzyme in the body than what was originally discovered. These "off target interactions" may be minimal, but they may also be enough to cause an effect in the body, allowing the potential use of these already approved agents as surrogate drugs for diseases that may not have safe and effective treatments available. The main purpose of this *in silico* docking study was to see if five different FDA approved drugs (Memantine, Levodopa, Tetrabenazine, Gabapentin, Zolpidem) were binding to different targets in the body that they were not originally discovered to bind. The targets selected for the *in-silico* docking studies were the 5HT5 receptor, 5HT4 receptor, α7 nicotinic acetylcholine receptor, α4β2 nicotinic acetylcholine receptor, D4 receptor, monoamine oxidase A (MAO-A), monoamine oxidase B (MAO-B), $GABA_A$, $GABA_B$, and Calcium-sensing receptor (CaSR).

The FDA approved drugs that were being selected for this study have been approved to treat neurodegenerative diseases. Diseases in this class include Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis, amyotrophic lateral sclerosis, Spinal muscular atrophy (SMA), and Huntington's disease. Neurodegenerative diseases destroy the structure and function of the central nervous system and peripheral nervous system. These types of diseases are important to study because the world's population is aging as modern medicine is advancing. Neurodegenerative diseases typically occur mid to late in life. It is expected that by 2030, around 12 million Americans will suffer from a neurodegenerative disease as 1 in 5 Americans will be over the age of 65 (1). This making the urgency to discover better targets for neurodegenerative disease more important. The diseases that this project looked at were Alzheimer's disease, Parkinson's disease, Huntington's disease, and spinocerebellar ataxia. Thus, the targets selected above for the *in-silico* studies have been found to be directly associated to these diseases discussed below or potentially related.

Alzheimer's Disease. Approximately 6.2 million Americans are currently living with Alzheimer's Disease (AD). This number is expected to rise to 12.7 million by 2050, if a preventative drug is not discovered by then (2). AD is characterized by a consistent decline in cognition over time. The loss of neurons typically starts in the hippocampus, basal forebrain and temporal and frontal cortex. Overtime, the atrophy of brain tissue is seen throughout the entire brain (2). The disease is associated with an increase in plaques and neurofibrillary tangles. Amyloid beta accumulation in vulnerable neurons is seen prior to plaque formation. The accumulation leads to neuronal cell lysis, which will then lead to plaque formation. It was hypothesized that these tangles and plaques are contributing to the progression of the disease (3). There are currently no drugs that target these plaques. Drugs that are currently approved for AD are those that modify symptoms only. This project looked at the drug memantine (Namenda), an uncompetitive NMDA receptor antagonist. This drug is thought to decrease excessive glutamate amounts, which may help with the learning and memory. Memantine has a reported Kd value of 7.1 μM for the NR1a/2B NMDA receptor (4).

Parkinson's Disease. Parkinson's Disease (PD) affects around one million people in the United States (5). PD is characterized by a loss of dopaminergic neurons in the substantia nigra (6). Dopamine acts as

an inhibitory neurotransmitter of GABA neurons (7). The excess GABA is thought to contribute to an imbalance of neurotransmission in the basal ganglia, which causes the motor symptoms associated with PD. Symptoms of PD will typically show when 60-80 percent of dopamine neurons are gone in the patient (8). Common treatments for this disease include increasing dopamine levels in the patients. Levodopa is a common treatment for PD. This drug works by increasing dopamine levels in the brain. The additional level of dopamine stimulates remaining dopamine receptors in the brain, D1 and D2. Another drug that may treat PD is zolpidem. Researchers discovered that this drug may have beneficial effects on motor symptoms associated with PD (9).

Huntington's disease. Huntington's disease affects 41,000 people in the United States, with 200,000 at risk of developing the disease (10). This disease is caused by a mutation in the huntingtin gene, which has a higher repetition of the codon CAG. The mutation causes the development of the protein called Huntington. This protein has an expanded region of polyglutamine residues, which will then accumulate in neurons and will lead to symptoms of the disease. Characteristics of the disease include atrophy of the caudate nucleus, spiny neurons in the corpus striatum die off and GABA neurotransmitters and substance P decrease. Symptoms of the disease have both psychiatric components and motor function abnormalities. There is no cure for this disease. The drugs used to help patients with this disease are only ones that alleviate symptoms. Tetrabenazine, a vesicular monoamine transporter type 2 (VMAT-2) inhibitor, is used to help chorea and dyskinesias symptoms (11).

Spinocerebellar ataxia. Spinocerebellar ataxia (SCA) is caused when the cerebellum and sometimes the spinal cord are damaged. There are multiple types of SCA and are classified by the mutated gene that causes SCA. Symptoms of this disorder include difficulty walking, lack of hand-eye coordination, abnormal speech (12). Two drugs that are prescribed for this disorder are Zolpidem and Gabapentin. Zolpidem is a $GABA_A$ receptor agonist (13). Gabapentin inhibits the alpha 2-delta subunit of the voltage-gated calcium channels (14). Zolpidem mildly helps ataxia and tremors (15). Gabapentin also mildly helps patients with SCA (16).

The different neurological disorders discussed above have been associated to different target and some of these targets have been selected for this study. A brief description of these targets is presented below.

5HT4 receptor. The 5HT4 receptor is located on neurons in the central nervous system (CNS), and is expressed in the basal ganglia, hippocampus and the neocortex (17). This receptor is thought to be involved with memory and learning. It has also been hypothesized that this receptor may be able to promote the non-amyloidogenic pathway (18). The receptor acts differently in the peripheral nervous system (PNS) as it aides in the release of acetylcholine in smooth muscle tissue of the intestinal tract. The receptor may play a role in gastroesophageal reflux disease (GERD) and irritable bowel syndrome (IBS) (19). Drugs targeting this receptor treat GERD and enhance gastric emptying such as Mosapride citrate (Kd value of 30nM) (20), which is an agonist for the 5HT4 receptor. Similarly, Prucalopride (prudac) also has a high affinity for the 5HT4 and has a Kd value of 8.7nM (21).

5HT5 receptor. The 5HT5 receptor family consist of two members, $5HT5_A$ and $5HT5_B$. $5HT5_B$ has not been found in humans (22). This receptor is expressed in the raphe nuclei, the cerebral cortex. This

receptor may be involved in autoreceptor function, sleep, exploratory behavior (23), and locomotion (24). Studies with mice have discovered that decreased expression of 5HT5a results in a significant impact in motor activity. When the mice are given a 5HT5a partial agonist, motor activity is decreased (24). This suggests that an antagonist of this receptor could help patients that suffer from a movement disorder such as SCA, Huntington's disease and Parkinson's disease. Partial agonist of this receptor includes LSD (Ki: 1.8nM), and (Ki:10.7 μM), which is used for insomnia (25). Agonists include 5-carboxamidotryptamine (5-CT) (Ki: 15.9 nM) (24). Antagonist of this receptor include Asenapine (Ki: 1.6 nM), which is used as a treatment for schizophrenia (26); Dimebon (Ki: 55nM), which is used to enhance cognition in patients that suffer from AD or Huntington's disease (27); and Ritanserin (Ki: 71nM). Studies on Ritanserin show that it may be used to help reduce daytime sleepiness (28).

α7 nicotinic acetylcholine receptor. α7 nicotinic acetylcholine receptors in the CNS and PNS are involved with memory, learning, anxiety and modulate synaptic transmission (29). It is also involved in the termination of the parasympathetic nervous system and participates in the cholinergic anti-inflammatory pathways (30). Decreased expression of this receptor has been associated with Alzheimer's disease, schizophrenia, and pain (29). Specifically, for Alzheimer's disease, the early accumulation of amyloid beta is correlated with the upregulation of α7 nAChR; therefore, targeting α7 nAChR may prevent the cell lysis caused by accumulated amyloid beta. Furthermore, stimulation of the α7 nAChR may protect neurons from amyloid beta degeneration. Thus, increasing expression of this receptor may prevent the progression of Alzheimer's disease and be used as a prevention tool to prevent the disease from forming (29). 5-(4-acetyl[1,4]diazepan-1-yl)pentanoic acid [5-(4-methoxyphenyl)-1H-pyrazol-3-yl] amide is agonist for this receptor with a reported Ki value of 660 nM. (31). W-56203 selectively bound to α7 nAChRs with a Ki value of 3 nM. This is a potential drug that could be used to treat schizophrenia (32). Both of these drugs are not FDA approved as there is not an approved drug on the market that modifies the progression of AD.

α4β2 nicotinic acetylcholine receptor. α4β2 nAChR is involved in cognition, mood, pain, and reward (33). Similar to the α7 nAChR, α4β2 nAChR has decreased expression during Alzheimer's disease (34). Varenicline is a partial agonist that binds selectively to the α4β2 nAChR over the α7 nicotinic acetylcholine receptor. The drug has a reported Ki value of 0.4nM (35). This drug is used for smoking cessation. Rivanicline is another drug that targets this receptor. It was developed for the treatment of AD. It has a reported Ki value of 25nM (36).

MAO-A: Monoamine Oxidase type A is an enzyme that breaks down monoamines, such as, serotonin, norepinephrine, epinephrine and dopamine. This enzyme shows a greater affinity for the neurotransmitter's norepinephrine and serotonin (37). Inhibitors of this enzyme have been thought to help with symptoms of psychiatric conditions characterized by a decrease in monoamines, such as anxiety and depression. The reasoning for this is that inhibitors will slow down the metabolism of monoamines, allowing more neurotransmitters to be available for neurotransmission (38). Bifemelane, an antipsychotic that is not FDA approved, is a competitive inhibitor of MAO-A with a Ki value of 4.20 μM (39). Clogyline is an irreversible inhibitor, Ki value 540nM. Moclobemide, reversible inhibitor and FDA approved, Kd value 200 μM (40).

MAO-B. Similar to MAO-A, MAO-B breaks down monoamines, but has a greater affinity for non-hydroxylated amines (37). MAO-B is specifically involved in the neurodegenerative process with aging. It was also seen to be involved at a greater level with PD and AD (41). MAO-B inhibitors have been hypothesized to have a neuroprotective effect because they would prevent dopamine metabolism and dopaminergic neuron degeneration. Preventing dopamine metabolism and/or dopaminergic neuron degeneration would decrease the symptoms of Parkinson's disease (42). Selegiline targets this enzyme and is an irreversible inhibitor with a Ki value of 29nM (43). Rasagiline is also an irreversible inhibitor with a Ki value of 700nM (44).

$GABA_A$. The GABA-a receptor control most of the inhibitory signaling in the CNS (45). It is a ligand-gated ion channel (46). It is involved with epilepsy treatment as activation of this receptor causes an increase in levels of chlorine (47). This increase causes cells to become hyperpolarized and less likely to have action potentials (48). Epilepsy is characterized by abnormal, synchronous firing of neurons; therefore, increasing inhibitory signaling may decrease the chance of a seizure (49). Drugs that target this receptor include benzodiazepines, barbiturates, neuroactive steroids and anesthetics (50). SR 95531 is a selective, competitive $GABA_A$ antagonist with a reported Ki value of 150nM (51).

$GABA_B$. Similar to $GABA_A$, this receptor is involved with inhibitory neurotransmissions. Unlike $GABA_A$ receptor, $GABA_B$ is a G-protein coupled receptor (GPCR) (52). Baclofen is a selective agonist for $GABA_B$ (53) and is used to treat spasticity, a condition where the muscle stiffen or tighten (54). The Ki value reported for this drug is 30 nM (55).

DRD4. The D4 receptor is a GPCR that is found in the CNS. This receptor has been targeted in an effort to create a treatment for PD, schizophrenia, depression, and attention deficit hyperactivity disorder (ADHD) (56). There are no D4 receptor specific ligands on the market, but clozapine is selective for the D4 receptor. This drug has a reported Ki value of 1.6 nM (57). Buspirone has a high affinity for DRD4 and a reported Ki value of 78 nM (58).

CaSR- the calcium sensing receptor is a GPCR that is involved in the homeostasis of calcium. The CaSR are expressed in the parathyroid gland. This receptor then controls the release of parathyroid hormone. Drugs that activate this receptor that are not calcium are called type 1 calcimimetics. These drugs are being researched in an effort to treat disorders that have an increase in parathyroid hormone (59). The result would be a decrease in the hormone and decrease calcium levels. Hyperparathyroidism is a disease characterized by increase in parathyroid hormone (60). Cinacalcet hydrochloride is a CaSR agonist used to treat this disorder. It enhances the activity of CaSR, which results in a decrease in calcium. However, this drug is known to have adverse side effects such as strong inhibition of CYP2D6 and nausea and vomiting (61). The Ki value of Cinacalcet was not found in a PubMed search. Ki value was then established using the same methods as the current study and it was found to be 102.97 nM.

Methods

Sample, target selection, and prioritization. A literature research of potential FDA approved drugs for neurological disorders was completed first (Figure 1). After the potential drugs were identified, a reference research looking at potential transporters and receptors was completed. Literature binding affinities of the drugs for their intended receptor or transporter was also noted. The results from preliminary *in-silico* docking studies were used to identify Ki values between the target and the FDA approved drug. Ki values are an inhibition constant used to describe the potency of an inhibitor (66). These values were compared to published Ki values of drugs that bind to the same targets. The Ki values that were prioritized were ones that were in a similar range of the published Kd values. The Ki and Kd values used in this study represent the level of binding affinity of a drug with a specific target.

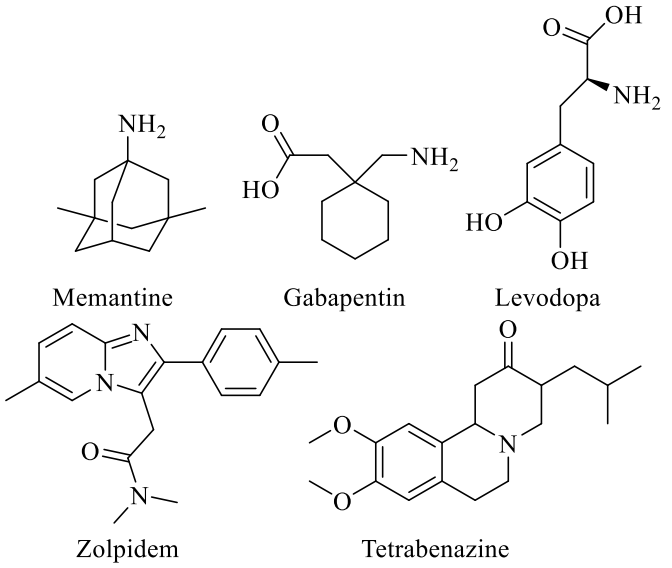

Figure 1. Chemical structures of drugs selected for study.

Protocol for *in silico* studies. Molecular docking between the FDA approved drugs memantine, levodopa, tetrabenazine, gabapentin, and zolpidem, and the targets (5-HT4, 5HT5, α7 nicotinic acetylcholine receptor, α4β2 nicotinic acetylcholine receptor, D4 receptor, MAO-A, MAO-B, GABAA, GABAB, CaSR) was performed. All *in silico* experiments used crystallographic structures from the Protein Data Bank website (PDB ID: 5HT4, 5HT5, 5AFM (α7 receptor), 5KXI (α4β2), 2Z5y (MOA-A), 4COF (GABA-A), 4MS3 (GABAB), 1GOS (MOA-B), 5WIV (DRD4), and 5K5S (CaSR)). These proteins were modified using Discovery Studio. Ligands and heteroatoms were deleted. The edited protein structure was inputted into Autodock Tools 1.5.6. Polar hydrogens were added and Kollman charges were assigned. Ligand was inputted and optimized with the protein in AutoDock. Default parameters were used in AutoDock, except the Genetic Algorithm Parameters were changed to 100 runs. The protein was set rigid, while the ligand was allowed to be flexible around the protein. The Grid Box size was adjusted to 126 Å × 126 Å × 126 Å in the x, y and z dimensions. The center of the grid corresponded to the different proteins and the chains that were analyzed. In all of the proteins used, one chain was selected to set the dimensions of the center grid box. The predicted docked protein-ligand complexes were based on the conformation with the lowest inhibition constant (Ki). Ki value was automatically calculated using the AutoDock Tools program that followed the equation ki=exp(ΔG ×1000/RT). Ki values were then compared to binding affinity values reported for the drugs that target the protein being analyzed.

Results and Discussion

Table 1-10 shows the Ki values of memantine, levodopa, gabapentin, tetrabenazine and zolpidem with the targets selected and the amino acids that the ligands are interacting with. The Ki values that were analyzed further were ones that were in a similar range to the literature Ki values of drugs and that are being reported to bind to this target for the first time. Ligand interactions with the drug that were within the literature Ki value range are shown in Figures 2-10.

Results from the *in-silico* studies between memantine, levodopa, gabapentin, tetrabenazine and zolpidem and 5HT4 target are presented in table 1 and figure 2.

Table 1. Protein-Ligand Interaction - 5HT4 (PDB ID: 5HT4)

FDA Approved Drugs	Inhibition Constant	Amino Acid
Memantine	1.64 µM	PHE-34, GLU-30, TYR-121, ILE-7
Levodopa	14.79 µM	GLU-183, TYR-162, GLU-161, LYS-132
Gabapentin	79.95 µM	GLU-30, THR-136, TYR-121, ILE-7 VAL-8, PHE134
Tetrabenazine	844.34 nM	LYS-18, ASP-21, TYR-121, ILE-7, THR-146m, ASP-145
Zolpidem	1.49 µM	TYR-121, ILE-7, SER-59, THR-56,

The Ki values found with the drugs used in the current study and 5HT4 (Table 1) have not been established yet. Based on this docking study, Tetrabenazine could be used to target the 5HT4 receptor in the CNS. This interaction would be a new finding as published research has not showed an interaction between tetrabenazine and 5HT4. Tetrabenazine has been discovered to influence serotonin syndrome by causing 5-HT release (68), but the exact interaction is not known. The cut off values used were in the nanomolar range as drugs used in a clinical setting that target this receptor are within the same range. Current drugs are targeting this receptor in the PNS in hopes of alleviating symptoms of GERD or IBS. These drugs do not readily cross the blood-brain barrier. The metabolite of tetrabenazine, dihydrotetrabenazine, does readily cross the blood-brain barrier in order to bind to the target in the CNS (64). This action may facilitate the drug to help enhance learning and memory to some degree as it already has capabilities to pass the blood brain barrier. Since the 5HT4 receptor is also involved in promoting the non-amyloidogenic pathway, it may be something that could be used in the treatment of Alzheimer's disease. Further studies would need to be completed to see if this drug, or an optimized version, would have a significant effect on learning, memory, and the non-amyloidogenic pathway. The effect tetrabenazine has on cognition and the non-amyloidogenic pathway have not been established yet.

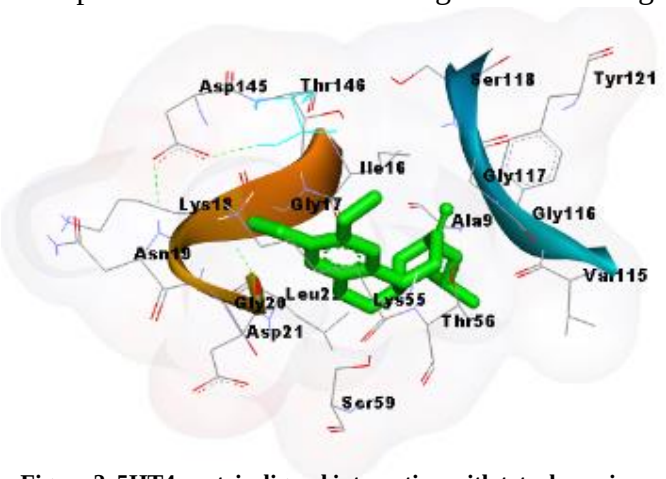

Figure 2. 5HT4 protein-ligand interaction with tetrabenazine.

Some 5HT4 receptor drugs have been discovered to have serious cardiac side effects and have even been pulled from the market, such as Cisapride. These older generation 5HT4 receptor agonist had interactions with the hERG potassium channel (16), which was causing the cardiac episodes. Tetrabenazine does not have common serious side effects. The most common side effects range from body aches to psychological symptoms such as depression (17). Since the side effects of tetrabenazine are not as severe, it may also be a beneficial treatment for GERD or IBS. Using tetrabenazine to treat GERD or IBS has not been established yet. Thus, further studies for a potential new application is highly encouraged.

The second target evaluated was 5HT5. Results from the *in-silico* studies between memantine, levodopa, gabapentin, tetrabenazine and zolpidem and 5HT5 target are presented in table 2 and figure 3.

Table 2. Protein-Ligand Interaction - 5HT5 (PDB ID:5HT5)

FDA Approved Drugs	Inhibition Constant	Amino Acid
Memantine	1.17 µM	TYR-121, ILE-7, GLU-30, PHE-34
Levodopa	71.29 µM	GLU-123, ARG-77, SER-76, SER-80, LEU-93, SER-119
Gabapentin	60.39 µM	GLU-30, TYR-121, ILE-7, PHE-34, THR-136, VAL-8
Tetrabenazine	632.44 nM	TYR-121, ILE-7, LYS-18, ASP-21, SER- 59, THR-136, VAL-8
Zolpidem	792.45 nM	LEU-22, ASP21, LYS-18, SER-59, THR-56m ILE-60

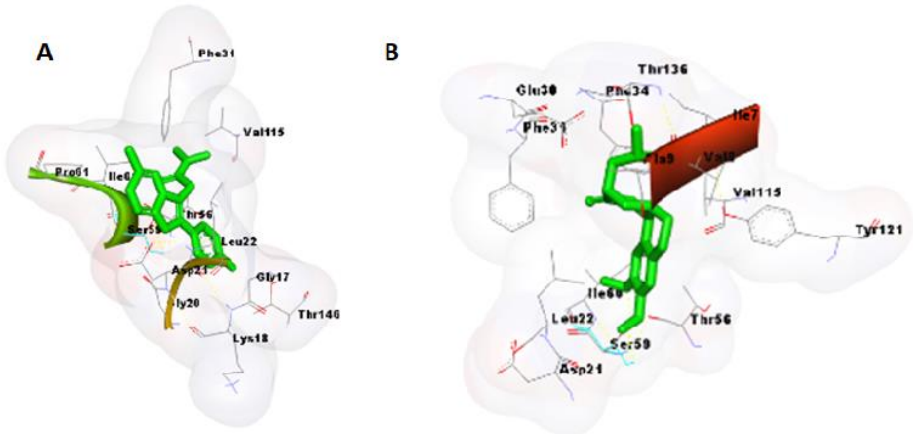

Figure 3. 5HT5 protein-ligand interaction with Zolpidem (A) and Tetrabenazine (B).

The Ki values found with the drugs used in the current study and 5HT5 (Table 2) have not been established yet. The docking study showed that tetrabenazine and zolpidem could be used to target the 5HT5a receptor, which exhibit the lowest K values. This interaction would be considered a new finding, suggesting that further studies could be of great value in the treatment of neurological disorders. The amino acid interactions are shown in Figure 3. Based on the literature Ki values from drugs known to target this receptor, the cut off values used were below 10 µM as the drug that had the lowest binding affinity was 10.7 µM (valerenic acid). The drugs that have been discovered for the 5HT5a receptor all treat disorders that are involved with the CNS, such as schizophrenia, enhancing cognition, and sleep disorders. Therefore, the drugs used in the current study could also be investigated to treat these disorders. Furthermore, many of the dugs that have been discovered to bind to this receptor are not FDA approved yet. These drugs include LSD, valerenic acid, 5-CT and Ritanserin. The drugs in the current study are FDA approved and may be a safe alternative to treat these disorders. Studies have shown that decreased expression of this receptor increase locomotion (21); therefore, antagonist of this receptor could help with movement disorders as well. Further studies

will need to be completed to determine if the drugs in the current study are antagonist, agonist or partial agonist.

The next target evaluated was α7 receptor. Results from the *in-silico* studies between memantine, levodopa, gabapentin, tetrabenazine and zolpidem and α7 target are presented in table 3 and figure 4.

Table 3. Protein-Ligand Interaction - α7 receptor (PDB ID: 5AFM)

FDA Approved Drugs	Inhibition Constant	Amino Acid
Memantine	2.73 µM	TRP-53, LEU-116, TYR-91, SER-144, TRP-145
Levodopa	29.12 µM	HIS-147, VAL-85, PRO-16, ASP-17, LEU-88, PRO-86
Gabapentin	44.27 µM	LEU-10, Leu-6, ASN-13, TRY-14, VAL-11, GLU-9, LYS-12, LYS-8
Tetrabenazine	758.94 nM	LEU-116, TRP-53
Zolpidem	1.93 µM	TYR-7, SER-77, THR-146, LEU-88, PRO-86, ILE-80

The Ki values found with the drugs used in the current study and α7 receptor (Table 3) have not been established yet. The docking study suggested new evidence that tetrabenazine may be able to target the α7 receptor. The cut-off values based on the literature Ki values were in the nanomolar range. The amino acid interactions are shown in Figure 4. Research suggest that the early pathophysiology of AD leads to the decreased expression, or destruction of the α7 nAChR. A drug that enhances the expression of this receptor would be considered a disease modifying drug. To date, there are no disease modifying drugs for AD that have received FDA approval. As tetrabenazine is already approved and has proven to be safe, it may be beneficial for this drug to be tested in the prevention of AD. Further studies will need to be completed to see if tetrabenazine is an agonist or antagonist for this receptor. Antagonism of this receptor may lead to further progression of AD as research has shown that decreased expression of this receptor has been discovered to lead to cell lysis brought on by amyloid beta.

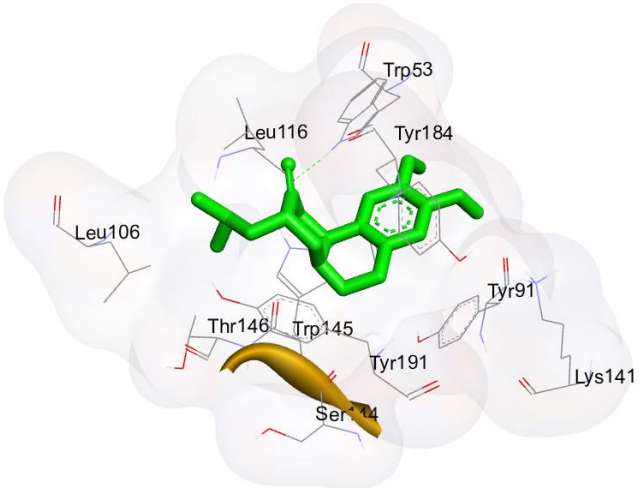

Figure 4. α7 nAChR protein-ligand interaction with tetrabenazine.

The fourth target evaluated was α4β2 receptor. Results from the *in-silico* studies between memantine, levodopa, gabapentin, tetrabenazine and zolpidem and α4β2 target are presented in table 4 and figure 5. The Ki values found with the drugs used in the current study and α4β2 receptor (Table 4) have not been established yet. The docking study showed that tetrabenazine and memantine may be able to target the α4β2 receptor.

Table 4. Protein-Ligand Interaction - α4β2 receptor (PDB ID: 5KXI)

FDA Approved Drugs	Inhibition Constant	Amino Acid
Memantine	374.89 nM	GLU-239, GLU-247
Levodopa	13.41 µM	GLN-46, LYS-147, ASN-60, TYR-102, ASN-97
Gabapentin	17.2 µM	GLU-268, TYR-212, LYS-53, THR,267, LEU-264
Tetrabenazine	258.44 nM	ARG-207, GLU-47, TRP-178, GLN-50, VAL-45
Zolpidem	2.26 µM	GLY-372, CYS-369, THR-287, TYR-283, LEU-376, VAL-236, THR-235

The cut-off values used for this determination were in the nanomolar range; however, the two drugs that were compared in the literature review that have been discovered to bind to this target, had a much smaller Ki value (0.4-25nM). The amino acid interactions are shown in Figure 5. Further optimization of tetrabenazine and memantine could make it more selective for the α4β2 receptor. Similar to α7 receptor, this receptor has decreased expression in AD. Since there are no FDA approved drugs that modify the progression of AD, this drug may be beneficial to try in patients that have AD as an alternative option once further studies confirm its potential use in this type of application. Furthermore, memantine is already indicated for symptom relief associated with AD. Further research would need to establish if this drug, as it is, acts as an agonist at this receptor. If optimized to interact with the α4β2 receptor more strongly, it may be a beneficial treatment for AD.

The next target evaluated was MAO-A. Results from the *in-silico* studies between memantine, levodopa, gabapentin, tetrabenazine and zolpidem and MAO-A target are presented in table 5 and figure 6.

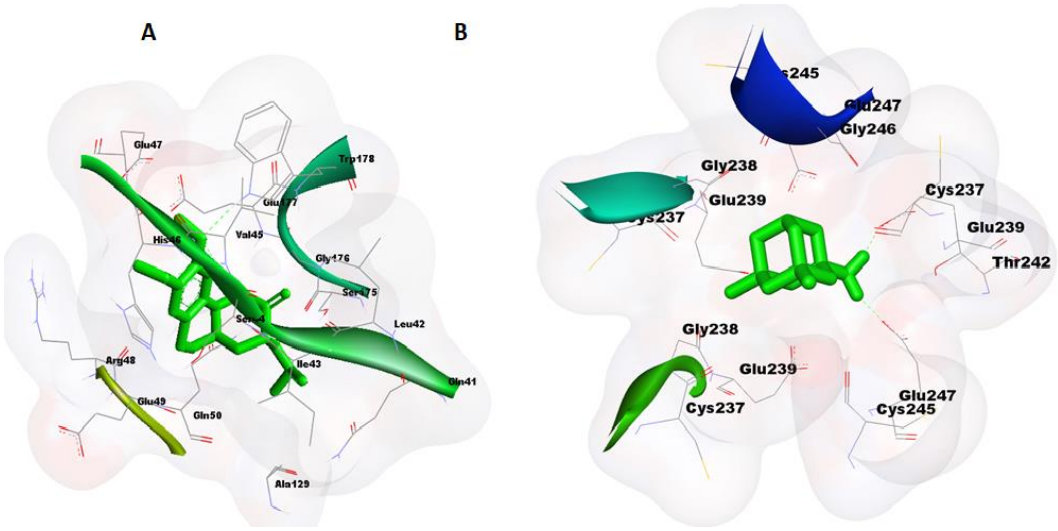

Figure 5. α4β2 nAChR protein-ligand interaction with tetrabenazine(a) and memantine (B).

The Ki values found with the drugs used in the current study and MAO-A (Table 5) have not been established yet. From the docking study, memantine, levodopa, gabapentin, tetrabenazine and zolpidem could be targeting the MAO-A enzyme. The drugs with the lowest values are tetrabenazine and zolpidem.

Table 5. Protein-Ligand Interaction - MAO-A (PDB ID: 2Z5Y)

FDA Approved Drugs	Inhibition Constant	Amino Acid
Memantine	5.11 µM	GLU-492
Levodopa	4.94 µM	GLN-401, ASP-46, TYR-402, ARG-47, GLY-404, GLU400, SER-403
Gabapentin	12.07 µM	ASP-46, ARG-47, GLY-50, LYA-395, GLU-400, ARG-45
Tetrabenazine	182.33 nM	ASP-328, ARG-356, GLU-185, LYS-357, LEU-176, ARG-172
Zolpidem	119.58 nM	GLY-67, ARG-51, LYS-305, GLY-66, ALA-68, TYR-69

Literature values for this receptor show Ki values in the micromolar range. All of the drugs that were studied had a Ki value in the micromolar range or nanomolar range. The protein-ligand interactions are shown below (figure 6). The drugs in the current study all pass the blood brain barrier where the MAO-A enzyme is located, so it could be possible that these drugs are substrates for this enzyme. Further research needs to be completed to establish what role these drugs have with this enzyme. If the drugs bind to this enzyme, then this may further explain these drugs' mechanism of action and its potential application in other related diseases. Furthermore, there are already effective treatments for disorders that are associated with a decrease in monoamines, but if these drugs were found to be potent inhibitors of this enzyme, then it may be beneficial to further study the effect these drugs have on disorders such as depression or anxiety.

The sixth target evaluated was GABA-A. Results from the *in-silico* studies between memantine, levodopa, gabapentin, tetrabenazine and zolpidem and GABA-A target are presented in table 6 and figure 7.

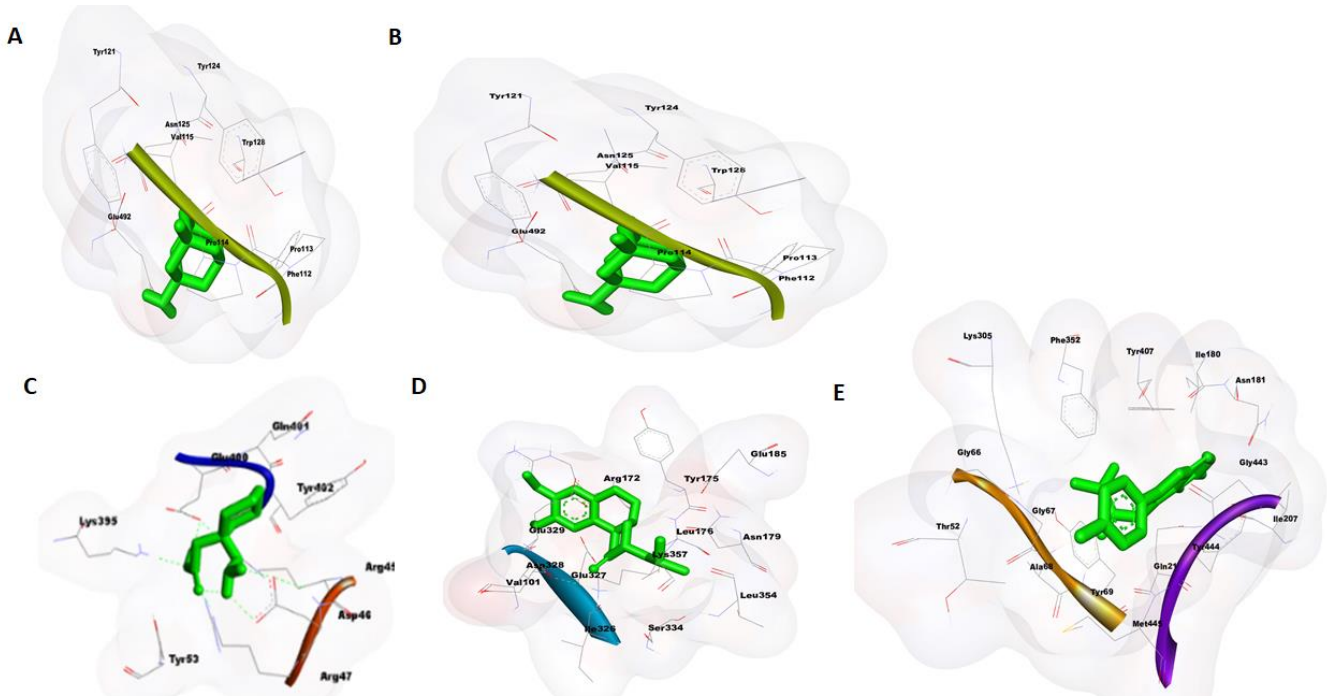

Figure 6. MAO-A protein-ligand interaction with memantine (a), levodopa (B), gabapentin (C), tetrabenazine (D), zolpidem (E).

The Ki values found with the drugs used in the current study and $GABA_A$ (Table 6) have not been established yet, with the exception of zolpidem. Based on the docking study, memantine could be used to target the $GABA_A$ receptor. The literature Ki value for a drug that binds to this receptor were in the nanomolar range; therefore, the cut-off value used was in the nanomolar range.

Table 6. Protein-Ligand Interaction – GABA-A (PDB ID: 4COF)

FDA Approved Drugs	Inhibition Constant	Amino Acid
Memantine	513.72 nM	TYR-97, TYR-62, GLU-155, ARG-117, TYR-205, THR-202
Levodopa	47.98 µM	GLU-182, ASP-48, CYS-136, MET-55, ILE-47, ASN-54, LYS-102
Gabapentin	17.05 µM	ARG-28, PRO-34, ASP-30, PRO-35, ARG-68, CYS-37
Tetrabenazine	1.15 µM	ILE-47, GLU-182, MET-138, MET-55
Zolpidem	1.66 µM	None Detected[1]

[1]Discovery studio did not detect any ligand interactions with amino acids.

Memantine had a Ki value of 513.72 nM (Table 6). The protein-ligand interactions are reported in figure 7. Research has elucidated that memantine mechanism of action is having antagonistic properties at the NMDA receptor. This antagonistic effect decreases glutamate. Glutamate is associated with having excitotoxic effects (62). If memantine binding to $GABA_A$ is confirmed, then it may be contributing to memantine's effect on patients that have AD. Furthermore, the activation of $GABA_A$ by memantine would help decrease the risk of seizures, if the drug acted as an agonist. However, if the drug was acting as an antagonist at the $GABA_A$ receptor, then it may cause an increased risk of seizures. Some research showed that memantine was actually associated with an increased risk of seizures (63). This could mean that memantine is acting as an antagonist at this receptor. Further research needs to be completed to understand the role memantine has at the $GABA_A$ receptor, if it has agonist or antagonist properties, and if this interaction is associated with an increased risk of seizures. The exact interaction of memantine has at the $GABA_A$ receptor is not known.

Zolpidem has been discovered to bind to the $GABA_A$ receptor with a Ki value of 13nM (65). The difference in reported Ki from the current study and the reported Ki value from the literature was most likely due to which subunit on the $GABA_A$ receptor Zolpidem was programmed to bind to.

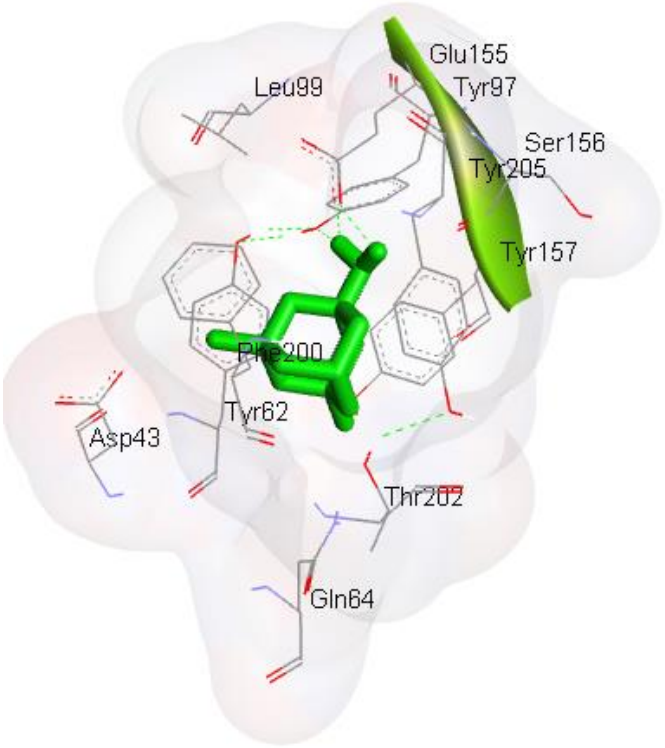

Figure 7. GABA-A protein-ligand interaction with memantine.

The next target evaluated using the selected drugs was GABA-B. Results from the *in-silico* studies between memantine, levodopa, gabapentin, tetrabenazine and zolpidem and GABA-B target are presented in table 7.

Table 7. Protein-Ligand Interaction – GABA-B (PDB ID: 4MS3)

FDA Approved Drugs	Inhibition Constant	Amino Acid
Memantine	1.9 µM	GLN-434, GLY-304, LYS-450, TYR-271, GLY-268
Levodopa	5.13 µM	ASP-411, ARG-416, SER-172, GLY-414, ARG-168, SER-158, SER-154
Gabapentin	8.94 µM	GLU-423, LYS-432, ASP-281
Tetrabenazine	2.76 µM	GLN-196, SER-225, ASN-234, ASP-207, THR-203, SER-233, GLU-210
Zolpidem	1.66 µM	SER-326, ASN-314, ALA-350, PHE-347

The Ki values found with the drugs used in the current study and $GABA_B$ (Table 7) have not been established yet. Based on the literature Ki values of the $GABA_B$ receptor, none of the drugs used in the current study appear to be significantly binding to the receptor with a similar binding affinity. Ki values for this receptor were values found in the nanomolar range based on the literature review. All of the drugs in the current study were in the micromolar range (Table 7). Thus, no figure of the binding results have been included.

The eighth target evaluated was MAO-B. Results from the *in-silico* studies between memantine, levodopa, gabapentin, tetrabenazine and zolpidem and MAO-B target are presented in table 8 and figure 8.

Table 8. Protein-Ligand Interaction – MAO-B (PDB ID: 1GOS)

FDA Approved Drugs	Inhibition Constant	Amino Acid
Memantine	2.01 µM	ILE-199, CYS-172, PHE-168
Levodopa	28.13 µM	GLY-12, GLU-34 ALA-35, GLY-11, ARG-36, GLU-34, HIS-273, LYS-271
Gabapentin	30.7 µM	LYS-81, ARG-208, GLU-207, ASN-203, GLN-475
Tetrabenazine	93.08 nM	GLN-206, ILE-198, SER-59, LYS-296, GLY-57, TYR-60
Zolpidem	124.6 nM	SER-59, GLY-58, ARG-42, GLN-206, ILE-198, LYS-296-GLY-57

The Ki values found with the drugs used in the current study and MAO-B (Table 8) have not been established yet. Based on the docking study, tetrabenazine and zolpidem could be used to target MAO-B. The cut-off values based on published Ki values for drugs that have been discovered to target this receptor are in the nanomolar range. Tetrabenazine and zolpidem both were found to exhibit Ki values in the nanomolar range (Table 8). The protein-ligand interactions of the two drugs are reported in figure 8.

Although controversial, some scientists believe that low-dose zolpidem may help neutralize the symptoms of Parkinson's disease (9). If zolpidem is having an interaction with MAO-B, then it may help explain why zolpidem neutralizes symptoms in some patients that have PD.

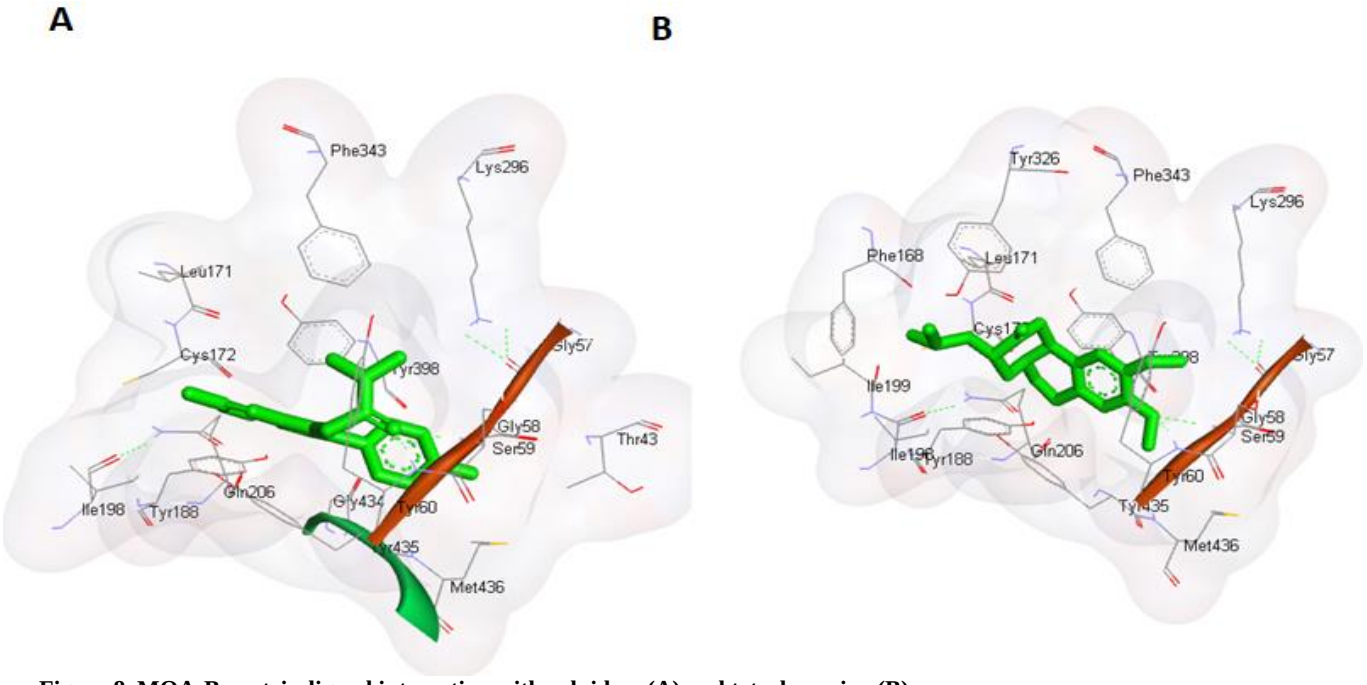

Figure 8. MOA-B protein-ligand interaction with zolpidem (A) and tetrabenazine (B).

As mentioned in the discussions of the acetylcholine nicotinic receptors, tetrabenazine may have an interaction with both receptors discussed. That interaction could be beneficial in the treatment of AD. If this drug also has an interaction at the MAO-B enzyme, then the need to further research tetrabenazine's role in AD seems more compelling as inhibition of MAO-B may have a neuroprotective effect (39). Further research needs to be done to establish if these two drugs are inhibitors of this enzyme.

The next target evaluated was DRD4. Results from the *in-silico* studies between memantine, levodopa, gabapentin, tetrabenazine and zolpidem and DRD4 target are presented in table 9 and figure 9.

The Ki values found with the drugs used in the current study and DRD4 (Table 9) have not been established yet. The docking study shows that zolpidem could potentially target the D4 receptor.

Table 9. Protein-Ligand Interaction – DRD4 (PDB ID: 5WIV)

FDA Approved Drugs	**Inhibition Constant**	**Amino Acid**
Memantine	11.04 µM	THR-449, PRO-445, PHE-459, PHE-455, VAL-44, GLY-51
Levodopa	38.5 µM	GLN-152, ASP-132, VAL-139, LEU-141, ARG-151, TYR-143
Gabapentin	78.72 µM	LYS-1047, GLU-1057, ALA-138
Tetrabenazine	1.11 µM	LEU-1106, ILE-1102, ILE-385, TYR-1105, HIS-1063, ASP-1060
Zolpidem	977.06 nM	LEU-1106, ILE-1102, ILE-385, TYR1105, PHE1061, GLU-1057

The literature Ki values found for this receptor were in the lower nanomolar range (1.6-78nM). The protein-ligand interactions are shown in figure 9.

There is some evidence that zolpidem may neutralize symptoms of Parkinson's disease in some patients. The interaction could potentially be due zolpidem having an agonistic relationship with the D4 receptor, which would cause the activation of the D4 receptor. As Parkinson's disease is characterized by the degeneration of dopamine receptors, this action would be therapeutic. Further research needs to be completed in order to establish what type of interaction zolpidem has at the D4 receptor.

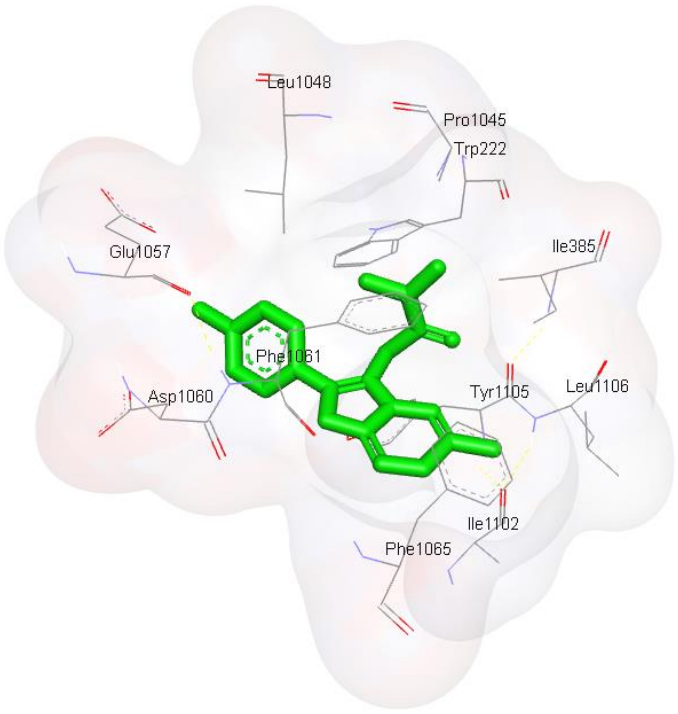

Figure 9. DRD4 protein-ligand interaction with zolpidem.

The last target selected for analysis was Calcium Sensing Receptor (CaSR). Results from the *in-silico* studies between memantine, levodopa, gabapentin, tetrabenazine and zolpidem and CaSR target are presented in table 10 and figure 10.

The Ki values found with the drugs used in the current study and CaSR (Table 10) have not been established yet. The cut-off values for CaSR were based on the docking study with Cinacalcet (Ki: 102.97 nM). Based on that Ki value, memantine could be binding to the calcium sensing receptor to produce an affect. The amino acid interactions are shown in figure 10.

Table 10. Protein-Ligand Interaction – CaSR (PDB ID: 5K5S)

FDA Approved Drugs	Inhibition Constant	Amino Acid
Memantine	377.05 nM	GLU-224, ARG-220, ALA-213, GLU-241, GLU-228
Levodopa	16.13 µM	HIS-413, ARG-415, THR-412, ARG-227, ARG-220
Gabapentin	11.26 µM	ARG-227, GLU-224, ARG-220
Tetrabenazine	277.34 µM	ARG-66, ARG-69, PRO-407, SER-417, TRP-70, ILE-416
Zolpidem	1.47 µM	ARG-465, GLN-49, GLY-483, ASP-480, LYS-181

If this drug acts as an agonist, then it may be a useful alternative to treat hyperparathyroidism. Cinacalcet is a potent CYP2D6 inhibitor (61), which may be problematic if a patient is taking drugs that are metabolized by the CYP2D6 enzyme.

Memantine is not a CYP2D6 inhibitor; however, memantine is a relatively potent CYP2B6 inhibitor (66), making this problematic if the patient is taking drugs that are metabolized by CYP2B6. Both drugs inhibit a cytochrome P450 enzyme. This is important to know because if a patient is taking a drug that inhibits the metabolism of another drug, then it can cause a drug to remain in the active form longer and could cause toxicity. If the drug is a prodrug, then it can lead to the drug never being converted to the active form. This will decrease the efficacy of the drug (67). The data obtained from the current study suggest new evidence that memantine could be used as an alternate treatment for those that suffer from hyperparathyroidism. Patients may be able to take memantine if they are taking drugs that require CYP2D6 metabolism. Further research will need to be completed to see if this drug acts as an agonist at this receptor and to optimize for this promising potential application.

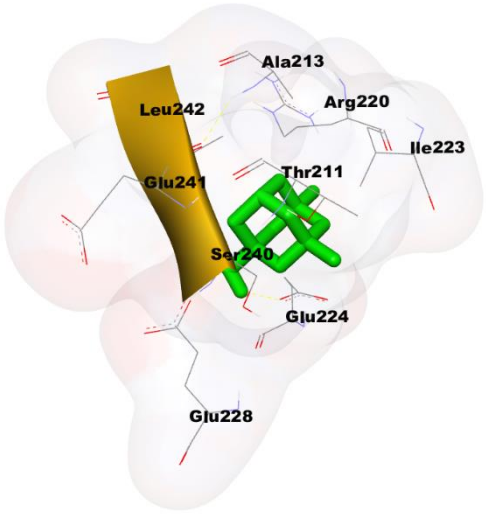

Figure 10. CaSR protein-ligand interaction with memantine.

Conclusions

The five drugs selected for analysis in this preliminary study have shown some type of interaction with a different target that was different to what it is expected to bind to. One of the drugs, memantine, exhibited interactions with at least three targets and showed Ki values in nanomolar range. Thus, the interactions that were deemed significant for the selected drugs need to be researched further to ensure that the interaction is not contributing to side effects of the drug, or if the interactions in this study are part of the drug's mechanism of action. Further analyses of these drugs can help optimize the drugs to treat not only the disorder it was indicated to treat more effectively, but also to optimize these drugs for potential new applications. Some of these drug-target interactions may also be significant enough to treat different disorders besides the disorder it was originally indicated to treat. Further *in vitro*, *in vivo*, and clinical experiments need to be completed in order to establish what beneficial or negative relationship these drugs have at the target they interacted with in this preliminary *in silico* studies.

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